Conservation biology of the critically endangered red-headed wood pigeon Columba janthina nitens in disturbed oceanic island habitats(Digest_要約)

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Summary

Chapter 1

Oceanic island ecosystems are vulnerable to human disturbances and many of island endemics are at risk of extinction. Conservation of endemic species on oceanic islands is one of the most pressing issues in order to reduce the rate of biodiversity loss, but their long-term conservation planning is not conducted based on sufficient scientific data. Recent development of molecular biological techniques may enable us to understand ecology and genetic characteristics of island endemics, and to suggest more concrete conservation planning. I investigated the critically endangered red-headed wood pigeon *Columba jantnia nitens*, an endemic subspecies which are living highly isolated and disturbed oceanic island, the Ogasawara Islands. This chapter introduce the aims and overview of the study; genetic characteristics and foraging ecology of the red-headed wood pigeon, suggesting its appropriate conservation plan within and outside of the pigeons' habitat, as a case study of endangered island endemics.

Chapter 2

I developed seven new microsatellite markers for two subspecies of the Japanese wood pigeon *Columba janthina*, the nominate Japanese wood pigeon *C. janthina janthina* and the red-headed wood pigeon *C. janthina nitens*. I also confirmed the cross-use of one microsatellite marker developed for *Columba livia* var. *domestica*. Seven loci were polymorphic in the nominate Japanese wood pigeon, while two loci were polymorphic in the red-headed wood pigeon. Using the markers, I performed a preliminary analysis of genetic diversity and genetic structure within each subspecies. The expected heterozygosity ranged from 0.00 to 0.64 in the nominate Japanese wood pigeon and from 0.00 to 0.08 in the red-headed wood pigeon. Each subspecies and each population within the nominate Japanese wood pigeon had different allele frequencies. The red-headed wood pigeon exhibited far lower genetic diversity than the nominate Japanese wood pigeon. Furthermore, the red-headed wood pigeon appears to have experienced a strong genetic drift from a common ancestral population, inferred by STRUCTURE analysis. The markers described here may be useful for investigating genetic diversity and genetic structure of Japanese wood pigeon populations, and could be used to estimate appropriate evolutionarily significant unit and to guide development of a captive

breeding program based on the genetic information.

Chapter 3

I conducted genetic analyses of the red-headed wood pigeon *Columba janthina nitens* using the DNA sequences of a portion of the mitochondrial control region and five microsatellite markers to estimate the genetic characteristics of the two wild populations from the Bonin and Volcano Islands, as well as one captive breeding population. The genetic diversity of the wild individuals was exceptionally low in both the mitochondria (nucleotide diversity = 0.00105) and at the microsatellite (3.2 alleles per locus and H_E = 0.12) loci. Higher numbers of microsatellite genotypes were observed in the Volcano Islands population than in the Bonin Islands population, which maybe because of the relatively low impact of human disturbance in the Volcano Islands. The most common mitochondrial haplotypes and microsatellite alleles observed in the two wild populations were completely fixed in the captive population. These results suggest that the genetic diversity of the captive population needs to be increased. However, introduction of a wild individual into a captive population can lead to a decreased genetic diversity in the small wild population and therefore should be done with caution. The genetic differentiation between the Bonin and the Volcano island groups was low, and the populations of the two island groups should be regarded as a single evolutionarily significant unit. However, special consideration is required for habitat conservation in the Volcano Islands, which may be functioning as a sanctuary for the red-headed wood pigeon. For the long-term conservation of threatened bird species that live on remote oceanic islands, determination of management units considering gene flows dependent on their flying capacity and maintenance of genetically suitable wild and captive populations are essential.

Chapter 4

Knowing the diet of an endangered animal is important for adequate nature restoration on oceanic islands. This is because the eradication of introduced species may fatally affect the endangered species when the endangered species rely on the introduced species as diet. DNA barcoding techniques together with high-throughput sequencing (HTS) may provide more detailed information on animal diets than other traditional methods. I performed a diet analysis using 48 fecal samples from the critically endangered red-headed wood pigeon *Columba janthina nitens* based on chloroplast *trn*L P6 loop sequences. The frequency of each detected plant taxon was compared with a micro-histological analysis of the same sample set.

The HTS approach detected a much larger number of plants than the micro-histological analysis. Plants those were difficult to identify by micro-histological analysis after being digested in the pigeon stomachs were frequently identified only by HTS. The results of HTS analysis indicated the frequent consumption of introduced species, in addition to several native species, by the red-headed wood pigeon. The rapid eradication of specific introduced species may reduce the food resource available to this endangered bird, and thus special consideration of pigeons' diet may be required in nature restoration planning. Although some technical problems still exist, the HTS approach may contribute to a better understanding of ecology of island endemics and their conservation.

Chapter 5

I studied the feeding ecology of the critically endangered red-headed wood pigeon *Columba janthina nitens*, a subspecies endemic to a highly remote and disturbed oceanic island chain: the Ogasawara Islands. An analysis based on high throughput sequencing (HTS) was carried out for 628 fecal samples collected over two years on two island habitats. Food availability and the nutrient composition of common fruit found on the two islands were also estimated. The results of the HTS diet analysis detected 122 food plant taxa and showed clear seasonal and inter-island variations in the pigeons' diet. Although the pigeons' preference for lipid rich fruits indicated, the pigeons' diet were flexibly changed according to food resource availability. This flexibility of food selection may reflect the foraging strategy of the pigeon in isolated island habitats with poor food resources. Pigeons also temporally consumed introduced plants in high frequency, which may complement the lack of preferable native food resources. The degree of the pigeons' dependence on introduced plants seems to differ between the two island habitats; thus, different impacts of the eradication of introduced plants on the pigeons' foraging condition on each island should be considered. HTS diet analysis combined with field data may be a useful tool to monitor the foraging conditions of endangered species, which may vary according to environmental changes, ecological disturbance and nature restoration in their habitat. This study may also contribute to an appropriate conservation in oceanic island ecosystems with complicated food webs including native and introduced species.

Chapter 6

This conclusion chapter summarizes how my study employing a multifaceted molecular biological approach revealed genetic characteristics and foraging ecology of the

red-headed wood pigeon, making it possible to inform conservation planning for the pigeon within and outside of the habitat. The results of this conservation biological study also suggested general management issues for island endemic birds: 1) careful selection of founder individuals for ex-situ conservation to maintain genetic diversity within and outside of the habitat 2) consideration of genetic structure, which may reflect flying capacity and may differ among species in detection of management units 3) nature restoration considering the interaction between native and introduced species, which may be temporary and/or spatially different. Further technical improvements of molecular biological methods and combination with field works may contribute appropriate conservation planning of the valuable but disturbed oceanic island ecosystems.

Appendix1

I performed genetic analyses, using 11 polymorphic microsatellite markers for six breeding colonies of the black-footed albatross *Phoebastria nigripes* on the Bonin Islands, an important breeding area in the western North Pacific, to determine appropriate management unit for long-tem conservation of genetic diversity. The results of AMOVA, STRUCTURE analysis, principal coordinates analysis, values of F_{ST}, R_{ST} and the migration rates suggested the lack of genetic differentiation aomong subpopulations. Although albatross species are strongly philopatric, the present results indicate a lack of population genetic differentiation among six subpopulations and the presence of sufficient gene flow to maintain the genetic homogeneity. In the principal coordinates analysis, a few individuals were genetically different from most of the other individuals, indicating a probability of immigration. The black-footed albatrosses on the Bonin Islands are in a good condition to maintain genetic diversity and can be treated as a single genetic management unit.

Appendix 2

To estimate possible gene flow caused by dispersal between populations two remote breeding sites: western North Pacific and central North Pacific, I performed genetic analysis on six colonies of black-footed albatross *Phoebastria nigripes* using 10 microsatellite markers. The central and western North Pacific populations were genetically differentiated. However, an estimation of migrants per generation indicated directional dispersal from the western to the central North Pacific.Compared to other Central North Pacific populations, the population on Kure Atoll, the westernmost atoll in the Hawaiian Islands in the central North Pacific, exhibited

weaker genetic differentiation from the western North Pacific populations, suggesting frequent immigration from the western North Pacific. The recent expansion of the western North Pacific population may be due to an increase in returning individuals, which may be caused by increased breeding success rates and /or survival rates. Range-wide and long-term monitoring of the black-footed albatross population using genetic markers may help to uncover dispersal dynamics of this highly mobile but philopatric albatross species and to make appropriate conservation decisions in light of environmental changes.

Appendix 3

Microsatellite markers were developed for the coastal shrub species *Scaevola taccada* to estimate the population genetic structure, which may reflect different seed dispersal patterns. Thirteen microsatellite primer sets were developed for *S. taccada* using 454 pyrosequencing. The primer sets were tested on 64 individuals sampled from two populations in Japan. Fragments were amplified using the primers, with one to 10 alleles per locus, and the expected heterozygosity ranged from 0.00 to 0.85. These results indicate the utility of markers in *S. taccada* for broad estimations of the population genetic structure of this species.